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TITLE: Immunological tolerance-inducing agent

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INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Holmgren; Jan	Vastra Frolunda	N/A	N/A	SE
Czerkinsky; Cecil	Goteborg	N/A	N/A	SE

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ABSTRACT:

An immunological tolerance-inducing agent comprising a mucosa-binding molecule linked to a specific tolerogen is disclosed. Further, a method of inducing immunological tolerance in an individual against a specific **antigen,** including hapten, which causes an unwanted immune response in said individual comprising administration by a mucosal route of an immunologically effective amount of an immunological tolerance-inducing agent of the invention to said individual, is described.

27 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Abstract Text - ABTX:

An immunological tolerance-inducing agent comprising a mucosa-binding molecule linked to a specific tolerogen is disclosed. Further, a method of inducing immunological tolerance in an individual against a specific **antigen,** including hapten, which causes an unwanted immune response in said individual comprising administration by a mucosal route of an immunologically effective amount of an immunological tolerance-inducing agent of the invention to said individual, is described.

Brief Summary Text - BSTX:

The present invention relates to an immunological tolerance-inducing agent.

Specifically it relates to such an agent comprising a mucosa-binding molecule linked to a specific tolerogen and to a method of inducing immunological tolerance in an individual against a specific **antigen,** including hapten.

Brief Summary Text - BSTX:

Introduction of a foreign substance, in the following referred to as **antigen** (Ag), including hapten, by injection into a vertebrate organism may result in the induction of an immune response characterized by the production of specific antibodies (products of B lymphocytes) capable of interacting with said Ag and/or the development of effector T lymphocytes and the production of soluble mediators, termed lymphokines, at the site of encounter with said Ag. Antibodies and T lymphocytes do certainly play an essential role in protecting against hostile Ag but can also participate in injurious processes leading to destruction of host tissues. This is the case in autoimmune diseases where antibodies and/or T lymphocytes react with Ag of one's own tissues and damage these. This is also the case in allergic reactions characterized by an exaggerated immune response to certain environmental matters and which may result in inflammatory responses leading to tissue destruction. Moreover, this is the case in chronic inflammatory reactions that develop as a result of ineffective elimination of foreign materials as in certain infections (e.g. tuberculosis, schisto-somiasis) or following introduction of foreign particles (e.g. asbestos). This is also the case in immunoproliferative reactions that follow the introduction into the body of an allograft and lead to its rejection.

Brief Summary Text - BSTX:

One of the primary goals in developing effective therapies against diseases caused by unwanted or tissue damaging immunological reactions such as allograft rejection, autoimmune diseases, tissue destructive allergic reactions to infectious microorganisms or to environmental **antigens,** is to specifically suppress or decrease to an acceptable level the intensity of deleterious immune processes without affecting the remainder of the immune system.

Brief Summary Text - BSTX:

The subject of immunological tolerance deals with all mechanisms that ensure an absence of destructive immune response, be it to one own's body constituents ("self **antigens**") or to any given foreign substance.

Brief Summary Text - BSTX:

A long-recognized method of inducing immunological tolerance is the oral administration of **antigen** which was first demonstrated by Wells for hen egg proteins (Wells, H. 1911. Studies on the chemistry of anaphylaxis III. Experiments with isolated proteins, especially those of hen's egg. J. Infect. Dis. 9:147). The phenomenon, often referred to as "oral tolerance" (because

initially documented by the effect of oral administration of Ag), is characterized by the fact that animals fed or having inhaled an **antigen** become refractory or have diminished capability to develop a systemic immune response when re-exposed to said Ag introduced by the systemic route, e.g. by injection. In broad terms, affication of an **antigen** onto a mucosal membrane or into a mucosal tissue, be it the intestine, the lung, the mouth, the genital tract, the nose or the eye, can induce the phenomenon of systemic immunological tolerance.

Brief Summary Text - BSTX:

As opposed to this, introduction of an **antigen** into a non mucosal tissue, i.e. for example the skin or the blood, referred to as systemic immunization, often results in an immune response with the characteristics mentioned above, and is referred to as systemic immune response.

Brief Summary Text - BSTX:

It is believed that ingested **antigens** are absorbed and processed by specialized cells, including epithelial enterocytes and Peyer's patch M cells, in the gut-associated lymphoid tissue (Owen, R. L., and P. Nemanic. 1978. **Antigen** processing structures of the mammalian intestinal tract: an SEM study of lympho-epithelial organs. Scanning Electron Microsc. 2:367-378.). It is also believed that inhaled **antigens** are uptaken by similar types of cells in the airway epithelium (Richardson, J., Bouchard, R. and Ferguson, C. C. 1976. Uptake and transport of exogenous proteins by respiratory epithelium. Lab. Invest. 35:307-314). Following interaction of the **antigen** with accessory cells and cognate helper T cells and/or B lymphocytes in the local micro-environment of the gut and of the lung mucosae, an immune response may ensue, the characteristics of which may be influenced by several factors, including the nature of the **antigen,** the type of accessory cells and lymphocytes involved, and the genetic background of the host. However, ingestion or inhalation of **antigens** may also result in the development of a state of peripheral immunological tolerance, a situation characterized by the fact that immune responses in non-mucosal tissues will not develop even if the **antigen** initially encountered in the digestive tract mucosa or the respiratory mucosa is reintroduced in the organism by a non-mucosal route, such as by parenteral injection. Since this phenomenon is exquisitely specific of the **antigen** initially ingested or inhaled, and thus does not influence the development of systemic immune responses against other **antigens,** its use has become an increasingly attractive strategy for preventing and possibly treating illnesses associated or resulting from the development of untoward and/or exaggerated immunological reactions against specific **antigens** encountered in non-mucosal tissues.

Brief Summary Text - BSTX:

The phenomenon of mucosally induced systemic tolerance may involve all types of immune responses known to be inducible by the systemic introduction of Ag, such

as the production of antibodies and the development of cell-mediated immune responses to said Ag. Mucosally induced immunological tolerance has therefore been proposed as a strategy to prevent or to reduce the intensity of allergic reactions to chemical drugs (Chase, M. W. 1946. Inhibition of experimental drug allergy by prior feeding of the sensitizing agent. Proc. Soc. Exp. Biol. 61:257-259). It has also been possible to prevent or decrease the intensity of immune reactions to systemically introduced soluble protein **antigens** and particulate **antigens** such as red cells in experimental animals and in humans by the oral administration of red cells (Thomas, H. C. and Parrot, D. M. V. 1974. The induction of tolerance to soluble protein **antigens** by oral administration. Immunology 27:631-639; Mattingly, J. and Waksman, B. 1978. Immunological suppression after oral administration of **antigen**. Specific suppressor cells found in rat Peyer's patches after oral administration of sheep erythrocytes and their systemic migration. J. Immunol. 121:1878; Bierme, S. J.; Blanc, M.; Abbal, M.; Fournie, A. 1979. Oral Rh treatment for severely immunized mothers. Lancet, 1:605-606).

Brief Summary Text - BSTX:

The phenomenon of mucosally induced systemic tolerance can be utilized to reduce or suppress immune responses not only against foreign **antigens** but also against self **antigens,** i.e. components derived from host tissues. It has thus been possible to decrease the intensity of experimentally induced autoimmune diseases in a variety of animal systems by mucosal deposition of autoantigens onto the intestinal (by feeding) or the respiratory mucosa (by aerosolization or intranasal instillation of **antigens**). Thus, oral administration of collagen type II (a prominent type of collagen found in joint cartilage) has been shown to suppress or decrease the intensity of experimental autoimmune arthritis, a disease that can be induced in certain strains of rodents by injection of collagen type II together with Freund's complete adjuvant or by injection of Mycobacterium tuberculosis (a component of the former adjuvant) alone (Thompson, H. S. G. and Staines, N. A. 1986. Gastric administration of type II collagen delays the onset and severity of collagen-induced arthritis in rats. Clin. Exp. Immunol. 64:581; Nagler-Anderson, C., Bober, L. A., Robinson, M. E., Siskind G. W., Thorbecke, G. J. 1986. Suppression of type II collagen-induced arthritis by intragastric administration of soluble type II collagen. Proc. Natl. Acad. Sci. USA 83:7443; Zhang, J. Z., Lee, C. S. Y., Lider, O. and Weiner, H. L. 1990. Suppression of adjuvant arthritis in Lewis rats by oral administration of type II collagen. J. Immunol. 145:2489-2493). Similarly, it has been possible to suppress an experimental form of autoimmune uveoretinitis by oral administration of S-**antigen,** a retinal autoantigen that can induce a form of uveoretinitis when injected in animals (Nussenblatt, R. B., Caspi, R. R., Mahdi, R., Chan, C. C., Roberge, R., Lider, O., Weiner, H. L. 1990. Inhibition of S-**antigen** induced experimental autoimmune uveoretinitis by oral induction of tolerance with S-**antigen**. J. Immunol. 144:1689-1695). Experimental autoimmune encephalitis, a chronic relapsing demyelinating disorder that can be induced in certain strains of rodents by injection of purified myelin basic protein or crude spinal cord homogenate together with adjuvant, can be suppressed partially or completely if animals are given MBP or MBP fragments by the oral (feeding) or respiratory (aerosol) route (Bitar, D. M. and Whitacre, C. C. 1988. Suppression of autoimmune encephalomyelitis by

the oral administration of myelin basic protein. Cell Immunol. 112:364; Higgins, P. J. and Weiner, H. L. 1988. Suppression of experimental autoimmune encephalitis by oral administration of myelin basic protein and its fragments. J. Immunol. 140:440-445; Weiner, H. L., Al-Sabbagh, A. and Sobel, R. 1990. **Antigen** driven peripheral immune tolerance: suppression of experimental autoimmune encephalomyelitis (EAE) by aerosol administration of myelin basic protein. FASEB J. (Abstr.) 4(7):2102). Furthermore, oral administration of insulin has been reported to suppress autoimmune diabetes in mice (Zhang, Z. J., Davidson, L., Eisenbarth, G. and Weiner, H. L. 1991. Suppression of diabetes in non obese diabetic mice by oral administration of porcine insulin. Proc. Natl. Acad. Sci. (USA) 88:10252-10256). More recently, suppression of experimental autoimmune myasthenia gravis has been achieved after oral administration of acetylcholine receptor (Wank, Z. Y., Qiao, J. and Link, H. 1993. Suppression of experimental autoimmune myasthenia gravis by oral administration of acetylcholine receptor. J. Neuroimmunol. 44:209-214).

Brief Summary Text - BSTX:

Much in the same way, oral administration of **antigens** has been proposed to prevent and/or treat allergic reactions to common allergens such as house dust components or substances present in grass pollen (Rebien W., Puttonen, E., Maasch, H. J., Stix, E. and Wahn, U. 1982. Clinical and immunological response to oral and subcutaneous immunotherapy with grass pollen extracts. A prospective study. Eur. J. Pediatry 138:341-344; Wortmann F. 1977. Oral hyposensitization of children with pollinosis or house dust asthma. Allergol et Immunopathol 5:15-26).

Brief Summary Text - BSTX:

Although the above examples indicate that mucosal administration of foreign as well as self **antigens** offers a convenient way for inducing specific immunologic tolerance, the applicability to large scale therapy in human and veterinary medicine remains limited by practical problems.

Brief Summary Text - BSTX:

Indeed, to be clinically broadly applicable, mucosally-induced immunological tolerance must also be effective in patients in whom the disease process has already established itself and/or in whom potentially tissue-damaging immune cells already exist. This is especially important when considering strategies of tolerance induction in patients suffering from or prone to an autoimmune disease, an allergic condition, or a chronic inflammatory reaction to a persistent microorganism. Current protocols of mucosally induced tolerance have had limited success in suppressing the expression of an already established state of systemic immunological sensitization (Hansson, D. G., Vaz, N. M., Rawlings, L. A. and Lynch, J. M. 1979. Inhibition of specific immune responses by feeding protein **antigens**. II. Effects of prior passive and active immunization. J. Immunol. 122:22612266).

Brief Summary Text - BSTX:

Most importantly, and by analogy with mucosal vaccines aimed at inducing immune responses to infectious pathogens, induction of systemic immunological tolerance by mucosal application of most **antigens** requires considerable amounts of tolerogen/antigen and, unless the tolerogen/antigen is administered repeatedly over long periods of time is of relatively short duration. A likely explanation is that most **antigens** are extensively degraded before entering a mucosal tissue and/or are absorbed in insufficient quantities. It has thus been widely assumed that only molecules with known mucosa-binding properties (examples of mucosa-binding molecules are listed in Table I below, see also reviews such as Mirelman, D. 1986. Microbial lectins and agglutinins, Properties and biological activity, pp. 84-110, Wiley, N.Y.) can induce local and systemic immune responses when administered by a mucosal route, such as the oral route, without inducing systemic immunological tolerance (de Aizpurua, H. J. and Russell-Jones, G. J. 1988. Oral vaccination. Identification of classes of proteins that provoke an immune response upon oral feeding. J. Exp. Med. 167:440-451). A notable example is cholera toxin, one of the most potent mucosal **immunogens** known so far (Elson, C. O. and Ealding, W. 1984. Generalized systemic and mucosal immunity in mice after mucosal stimulation with cholera toxin. J. Immunol. 132:2736) and which when administered simultaneously with an unrelated **antigen** by the oral route can also prevent induction of systemic immunological tolerance to said **antigen** (Elson, C. O. and Ealding, W. 1984. Cholera toxin did not induce oral tolerance in mice and abrogated oral tolerance to an unrelated **antigen**. J. Immunol. 133:2892).

Brief Summary Text - BSTX:

Based on these observations, mucosal administration of **antigens** coupled to mucosa-binding molecules such as cholera toxin or its mucosa-binding fragment cholera toxin B subunit, has been proposed as a strategy to induce local and systemic immune responses rather than systemic tolerance (McKenzie, S. J. and Halsey, J. F. 1984. Cholera toxin B subunit as a carrier protein to stimulate a mucosal immune response. J. Immunol. 133:1818-1824; Nedrud, J. G., Liang, X., Hague, N. and Lamm, M. E. 1987. Combined oral/nasal immunization protects mice from Sendai virus infection. J. Immunol. 139:3484-3492; Czerkinsky, C., Russell, M. W., Lycke, N., Lindblad, M. and Holmgren, J. 1989. Oral administration of a streptococcal **antigen** coupled to cholera toxin B subunit evokes strong antibody responses in salivary glands and extra-mucosal tissues. Infect. Immun. 57:1072-1077; de Aizpurua, H. J. and Russell-Jones, G. J. 1988. Oral vaccination. Identification of classes of proteins that provoke an immune response upon oral feeding. J. Exp. Med. 167:440-451; Lehner, T., Bergmeyer, L. A., Panagiotidi, C., Tao, L., Brookes, R., Klavinskis, L. S., Walker, P., Walker, J., Ward, R. G. et al. 1992. Induction of mucosal and systemic immunity to a recombinant simian immunodeficiency viral protein. Science 258(5036):1365-1369).

Brief Summary Text - BSTX:

As opposed to the established opinion that mucosal administration of **antigens** coupled to mucosa-binding molecules induce local and systemic immune responses, the present inventors have surprisingly found that **antigens** administered by various mucosal (oral, intranasal, vaginal, rectal) routes, when linked to a mucosa-binding molecule, enhanced induction of systemic immunological tolerance towards said **antigens**.

Brief Summary Text - BSTX:

The term "immunological tolerance" is here defined as a reduction in immunological reactivity of a host towards specific tolerated **antigen**(s). Such tolerated **antigen** is in the present specification and claims called a tolerogen, which is in agreement with established terminology.

Brief Summary Text - BSTX:

In another embodiment of the invention the specific tolerogen, which is linked to a mucosa-binding molecule in an immunological tolerance-inducing agent of the invention is selected from specific **antigens,** including haptens, which cause an unwanted immune response in an individual. In a preferred embodiment of the invention said **antigens** are selected from the group consisting of proteins, peptides, carbohydrates, lipids and nucleic acids.

Brief Summary Text - BSTX:

The way in which the specific tolerogen is linked to a mucosa-binding molecule in an immunological tolerance-inducing agent of the invention is not important as long as said tolerogen and said molecule can perform their respective function. Thus, they may be linked to each other directly by simple chemical procedures. Chemical procedures to couple proteins such as the B subunit of cholera toxin (CTB) or the thermolabile enterotoxin of Escherichia coli (LTB) to lipids, haptens, carbohydrates, nucleic acids as well as to other proteins including antibodies and synthetic peptides are well known in the art (e.g. see Carlsson, J. et al 1978. Biochem. J. 173:723-737; Cumber, J. A. et al. 1985. Methods in Enzymology 112:207-224; Walden, P. et al. 1986. J. Mol. Cell Immunol. 2:191-197; Gordon, R. D. et al. 1987. Proc. Natl. Acad. Sci. (USA) 84:308-312; Avrameas, S. and Ternynck, T. 1969. Immunochemistry 6:53; Joseph, K. C., Kim, S. U., Stieber, A., Gonatas, N. K. 1978. Proc. Natl. Acad. Sci. USA 75:2815-2819; Middlebrook, J. L. and Kohn, L. D. (eds): 1981. Receptor-mediated binding and internalization of toxins and hormones. Academic Press, New York, pp 311-350). The tolerogen can also be fused genetically to the CTB (or LTB) gene (Sanchez, J., Svennerholm, A-M and Holmgren, J. 1988. Genetic fusion of a non-toxic heat-stable enterotoxin-related deca-peptide **antigen** to cholera toxin B subunit. FEBS Letters 241:110-114) and the resulting chimeric gene then be expressed in a suitable expression system, such as a bacteria, a yeast or a virus. Alternatively, the tolerance inducing agent may comprise a fragment of a nucleic acid sequence (DNA or RNA) or a synthetic polynucleotide encoding the tolerogen which is then chemically coupled to the mucosa-binding molecule and administered by the mucosal route, advantage being

then taken of the capacity of cells from host mucosal tissues to ensure transcription and/or translation of the corresponding gene into a mature protein (Rohrbaugh, M. L. and McGowan, J. J. 1993. Gene-transfer for therapy and prophylaxis of HIV-1 infection. Ann. N.Y. Acad. Sci. Vol 685, pp 697-712; Nabel, G. J. and Felgner, P. L., 1993. Direct gene-transfer for immunotherapy and immunization. Trends in Biotechnology Vol 11 No. 5, pp 211-215; Robinson, H. L., Hunt, L. A., Webster, R. G. 1993. Protection against a lethal influenza-virus challenge by immunization with a hemagglutinin-expressing plasmid DNA. Vaccine 11:957-960; Martinon, F., Krishnan, S., Lenzen, G., Magne, R., Gomard, E., Guillet, J. G., Levy, J. P. and Meulien, P. 1993. Eur. J. Immunol. 23:1719-1722). Yet other alternative presentation forms could consist in the incorporation of the tolerogen or its nucleic acid precursor into a protective vehicle such as a liposome or equivalent biodegradable vesicles onto which the mucosa-binding substance had been or shall be attached allowing efficient binding of the tolerogen-containing vehicle to a mucosal surface for improved tolerogenic efficacy. With this type of presentation form, the tolerogen may be either free or linked to another molecule.

Brief Summary Text - BSTX:

The present invention is also directed to a method of inducing immunological tolerance in an individual against a specific **antigen,** including hapten, which causes an unwanted immune response in said individual comprising administration by a mucosal route of an immunologically effective amount of an immunological tolerance-inducing agent according to the invention to said individual. Examples of specific **antigens** which cause an unwanted immune response in an individual and which may form the specific tolerogen in the immunological tolerance-inducing agent of the invention are:

Brief Summary Text - BSTX:

a transplantation **antigen** or fragments thereof, including synthetic peptides or corresponding nucleic acid genetic information, or a cell expressing said transplantation **antigen,** such as a red blood cell, a platelet or a lymphocyte,

Brief Summary Text - BSTX:

and the agent of the invention can be administered by the mucosal route so as to prevent or reduce immune responses to said transplantation **antigen** and thus to prevent rejection and/or prolong survival of an allograft;

Brief Summary Text - BSTX:

The invention is exemplified by the use of CTB and of LTB as mucosa-binding molecules, and of sheep red blood cells (SRBC) and human gamma-globulins (HGG) as antigens/tolerogens. While the invention is in no way limited to tolerance induction against SRBC or HGG, these **antigens** are chosen as models of

particulate and soluble **antigens,** respectively, since they are among the best characterized oral tolerogens with regard to both antibody formation and cell-mediated immune reactions, the latter reactions being typified by the classical delayed type hypersensitivity (DTH) reaction. These types of immune reactions have been implicated in the development of autoimmune diseases, allergic reactions, graft rejection and other inflammatory conditions. The invention is further exemplified by the use of myelin basic protein which, when coupled to CTB and given by the oral route of administration, can suppress experimental autoimmune encephalitis, and by the use of allogeneic mouse thymocytes which, when coupled to CTB and given orally, can prolong allograft survival.

Brief Summary Text - BSTX:

SPDP-derivatized HGG and CTB were mixed at an equimolar ratio and incubated for 16 h at 23.degree. C. The resulting CTB-HGG conjugate was purified by gel filtration through a column of Sephacryl S-300 to remove free CTB and/or HGG. The resulting conjugate was shown to contain G.sub.M1 ganglioside binding capacity and to retain both CTB and HGG serological reactivities by means of an ELISA using G.sub.M1 (Sigma, St. Louis, Mo.) as solid phase capture system (Svennerholm, A.-M., and J. Holmgren. 1978. Identification of Escherichia coli heat-labile enterotoxin by means of a ganglioside immunosorbent assay (GM.sub.1 -ELISA) procedure. Curr. Microbiol. (1:19-23), and monoclonal and polyclonal antibodies to CTB and HGG as detection reagents (see below). Serial two-fold dilutions of the conjugate and of purified CTB- and HGG-SPDP derivatives were incubated in polystyrene wells that had previously been coated with GM1 ganglioside, and in wells coated with rabbit polyclonal IgG antibodies to HGG; next, horseradish peroxidase (HRP) conjugated rabbit ant-HGG or mouse monoclonal anti-CTB antibodies, appropriately diluted in PBS containing 0.05% Tween 20, and enzyme substrate were applied sequentially to detect solid phase bound HGG and CTB. The amount of free and bound HGG and CTB was determined by reference to standard curves calibrated with known amounts of SPDP derivatized **antigens**. On average, the SPDP conjugation procedure and purification protocol described above yielded preparations containing negligible amounts of free HGG and less than 10% free CTB.

Detailed Description Text - DETX:

To determine whether mucosal administration of CTB-conjugated **antigens** would suppress DTH reactions in animals previously systemically sensitized to said **antigen,** SRBC were first injected in the left rear footpad of mice to induce a state of primary systemic immunity. Four days later, animals were fed a single oral dose of SRBC conjugated to CTB, SRBC alone, or saline. Two days after the latter feeding, animals were given a second injection of SRBC in the right footpad to elicit DTH reactions. The latter DTH responses were monitored at various times after this secondary systemic immunization. Whereas mice fed SRBC alone developed DTH responses undistinguishable from those seen in control animals fed only saline, mice fed SRBC conjugated to CTB had considerably reduced early and late DTH responses to SRBC. Therefore, it appears that oral administration of SRBC conjugated to CTB can induce suppression of both early

and late DTH responses to systemically injected SRBC even in animals previously sensitized (primed) systemically to SRBC.

Detailed Description Text - DETX:

To determine whether oral administration of CTB-conjugated **antigens** would result in decreased proliferative responses of lymph node cells to said **antigens,** mice were fed a single dose of CTB-conjugated SRBC and were then injected in the left footpad with SRBC (primary systemic in vivo immunization). One week later, the ability of lymph node cells to proliferate after in vitro exposure to the homologous **antigen** (SRBC) was examined. Compared to control animals fed saline only and to animals fed a single dose of SRBC alone, lymph node cells from animals fed SRBC conjugated to CTB had decreased proliferative responses when cultured with SRBC (Table 4). This decrease was specific of the **antigen** administered in as much as the proliferative responses of lymph node cells to the mitogen concanavalin A were comparable in animals fed SRBC-CTB, SRBC or saline only (Table 4).

Detailed Description Text - DETX:

To determine whether oral administration of an **antigen** coupled to CTB would result in decreased antibody responses to systemically administered **antigen,** mice were fed a single dose of SRBC-CTB, SRBC alone, or saline which was given 1 to 8 weeks before a primary systemic immunization with SRBC injected in the left rear footpad. Five days after this injection, the right rear footpad was challenged and blood was collected from the tail vein 1 week later. Serum antibody levels to SRBC were determined by direct and indirect hemagglutination assays. As seen in Table 5, serum antibody responses to SRBC were decreased in animals fed a single dose of SRBC-CTB as compared to animals fed saline only or a single dose of SRBC alone. Daily oral administrations of SRBC for 3 weeks were required to suppress serum antibody responses to systemically administered SRBC to a level comparable to that achieved by a single administration of SRBC conjugated to CTB (Table 5).

Detailed Description Text - DETX:

To determine whether mucosal administration of CTB-conjugated **antigens** would suppress DTH reactions to a soluble protein **antigen,** mice were fed a single dose of HGG conjugated to CTB, HGG alone, or saline. These were given to separate groups of mice 1 week before a primary systemic immunization with HGG in Freund's complete adjuvant injected subcutaneously. Five days after this injection, the right rear footpad was challenged with HGG so as to elicit a DTH reaction. The intensity of DTH reactions elicited in mice fed 1 mg of HGG alone was comparable to that in control mice fed saline only, at all times examined after challenge (Table 7). Feeding mice 5 mg of HGG resulted in decreased DTH reactions at 24-48 hrs but did not influence the intensity of the early (2-4 hrs) phase of these reactions. In contrast, DTH reactions monitored in mice fed as little as 15 .mu.g of HGG conjugated to CTB, that is a more than 300-fold lower amount of HGG, had similar effects, being significantly lower

than corresponding reactions in control (saline fed) animals at 24 hrs, but not at earlier times (2 and 4 hrs). However, feeding mice with 66 .mu.g of HGG conjugated to CTB resulted in considerably decreased DTH reactions at all times recorded. Thus, the early (2-4 hr) and late (24-48 hr) DTH reactions were virtually abrogated in mice fed 66 .mu.g of HGG conjugated to CTB. These observations demonstrate that oral administration of small amounts of a soluble protein **antigen** conjugated to the mucosa-binding molecule CTB can induce suppression of both early and late DTH reactions to subsequent systemic injection with said protein **antigen**.

Detailed Description Paragraph Table - DETL:

TABLE 4 _____ Inhibition of **antigen** specific lymphocyte proliferation by oral administration of sheep red blood cells (SRBC) linked to the B subunit of cholera toxin (CTB) mean S. I. values .+-. 1 standard deviation in cultures exposed to feeding one dose of: SRBC concanavalin A _____ SRBC-CTB 1.06 .+-. 0.29* 119 .+-. 32 (n = 6 mice) SRBC 7.88 .+-. 4.52 108 + 56 (n = 6 mice) saline 8.94 .+-. 3.89 76 .+-. 35 (n = 6 mice)

_____ *denotes significant difference (P < 0.01; Student't test) between test SRBCCTB fed animals and animals fed SRBC alone or fed saline only.

Claims Text - CLTX:

1. A method of inducing immunological tolerance in a mammal to a T-cell-associated immunological response, which comprises administering by a mucosal route to a mammal suffering from or prone to a T-cell associated disease an immunological tolerance-inducing agent, wherein said agent comprises (i) a mucosa-binding molecule selected from the group consisting of the B subunit of cholera toxin and the B subunit of **heat-labile enterotoxin** of Escherichia coli, linked to (ii) a specific tolerogen associated with said T-cell associated immune response, and wherein said agent is administered in an amount and for a time effective to induce tolerance against said T-cell associated immune response.

Claims Text - CLTX:

8. A method as defined in claim 7, wherein said tolerogen is S-**antigen**.

Claims Text - CLTX:

14. A method as defined in claim 2, wherein said tolerogen is a transplantation **antigen**.

Claims Text - CLTX:

15. A method of inducing immunological tolerance in a mammal to diabetes, which comprises administering by a mucosal route to a mammal suffering from or prone to diabetes an immunological tolerance-inducing agent, wherein said agent comprises (i) a mucosa-binding molecule selected from the group consisting of the B subunit of cholera toxin and the B subunit of **heat-labile enterotoxin** of Escherichia coli, linked to (ii) a specific tolerogen comprising insulin, and wherein said agent is administered in an amount and for a time effective to induce tolerance against said diabetes.

Claims Text - CLTX:

16. A method of inducing immunological tolerance in a mammal to rheumatoid arthritis, which comprises administering by a mucosal route to a mammal suffering from or prone to rheumatoid arthritis an immunological tolerance-inducing agent, wherein said agent comprises (i) a mucosa-binding molecule selected from the group consisting of the B subunit of cholera toxin and the B subunit of **heat-labile enterotoxin** of Escherichia coli, linked to (ii) a specific tolerogen comprising cartilage-associated collagen, and wherein said agent is administered in an amount and for a time effective to induce tolerance against said rheumatoid arthritis.

Claims Text - CLTX:

26. An immunological tolerance-inducing agent for suppressing autoimmune diabetes, comprising a mucosa-binding molecule linked to insulin, wherein (i) said mucosa-binding molecule is the B subunit of cholera toxin or the B subunit of **heat-labile enterotoxin** of Escherichia coli and confers binding of said agent to mucosal cells and (ii) said agent suppresses autoimmune diabetes.

Other Reference Publication - OREF:

Mcl. Mowat, A., The regulation of immune responses to dietary protein **antigens,** Immunology Today, vol. 8, No. 3, 1987, pp. 93-98.

Other Reference Publication - OREF:

Czerkinsky, C., Russell, M.W., Lycke, N., Lindblad, M., and Holmgren, J., Oral Administration of a Streptococcal **Antigen** Coupled to Cholera Toxin B Subunit Evokes Strong Antibody Responses in Salivary Glands and Extramucosal Tissues, Infection and Immunity, vol. 57, No. 4, Apr. 1989, pp. 1072-1077.

Other Reference Publication - OREF:

Vives, J., Parks, D.E., and Weigle, W.O., Immunologic Unresponsiveness After Gastric Administration of Human .gamma.-Globulin: **Antigen** Requirements and Cellular Parameters, From the Department of Immunopathology, Scripps Clinic and research Foundation, La Jolla, CA, The Journal of Immunology, vol. 125, No. 4,

Oct. 1980, pp. 1811-1816.

Other Reference Publication - OREF:

Elson, C.O., and Ealding, W., Cholera Toxin Feeding Did OT Induce Oral Tolerance In Mice And Abrogated Oral Tolerance To An Unrelated Protein **Antigen,** From the Department of Medicine, Medical College of Virginia, Virginia Commonwealth University, Richmond VA, The Journal of Immunology, vol. 133, No. 6, Dec. 1984, pp. 2892-2897.

L Number	Hits	Search Text	DB	Time stamp
1	17	(heat-labile with II with enterotoxin)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/13 13:24
7	694	"I2" and subunit	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/13 13:06
13	914	"I1" and subunit	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/13 13:05
19	11	((heat-labile with II with enterotoxin)) and subunit	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/13 13:20
25	2	((heat-labile with II with enterotoxin)) and (salivary adj binding adj protein)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/13 13:21
31	3	heat-labile and (salivary adj binding adj protein)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/13 13:22
37	11	((heat-labile with II with enterotoxin)) and (immunogen or antigen)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/13 13:24
-	3	"5800821"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:07
-	2	"9958145"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:17
-	237	enterotoxin same vaccine	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:18
-	29	heat$labile with II	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:19
-	7	((enterotoxin same vaccine) and (heat$labile with II)) and subunit$	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:19
-	318	cholera adj toxin adj B	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:21
-	0	(cholera adj toxin adj B) same subunit$ same A2/B	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:21
-	245	(cholera adj toxin adj B) same subunit$	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:22
-	2	(cholera adj toxin adj B) same subunit$ same adjuvant same expression	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:22

-	1	(cholera adj toxin adj B) same subunit$ same adjuvant same plasmid	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:24
-	8	(enterotoxin same vaccine) and (heat$labile with II)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/13 13:04
-	62	(cholera adj toxin adj B) same subunit$ same adjuvant	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/02/26 18:32

FILE 'MEDLINE, BIOSIS, CAPLUS, EMBASE' ENTERED AT 15:20:27 ON 13 AUG 2002

L1	0	S HEAT-LABILE II ENTEROTOXIN
L2	4	S HEATLABILE ENTEROTOXIN
L3	18861	S HEAT LABILE
L4	4729	S L3 AND ENTEROTOXIN
L5	1306	S L4 AND SUBUNIT
L6	203	S L5 AND PLASMID
L7	4	S SALIVARY BINDING PROTEIN
L8	0	S L6 AND L7
L9	0	S L5 AND L7
L10	125	S L6 AND 1989-2000/PY
L11	71	DUP REM L10 (54 DUPLICATES REMOVED)
L12	18	S L11 AND ANTIGEN
L13	26	S L11 AND FUSION
L14	35	S L12 OR L13
L15	3867	S RUSSELL M?/AU OR CONNELL T?/AU
L16	18	S L15 AND L5
L17	5	DUP REM L16 (13 DUPLICATES REMOVED)
L18	40	S L14 OR L17